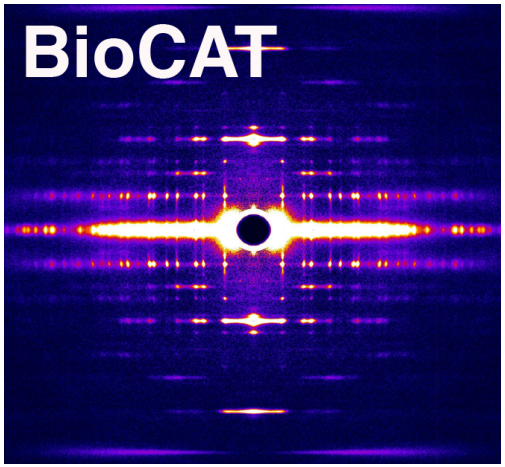
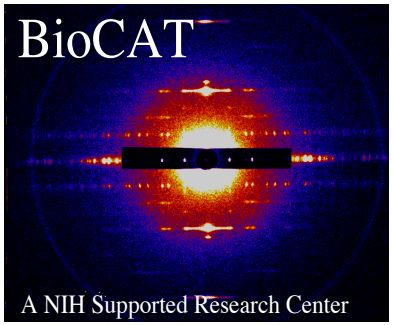
Non-Crystalline Diffraction and Scattering at the BioCAT Facility at the Advanced Photon Source



Tom Irving

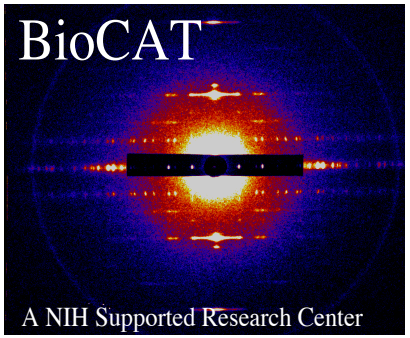
BioCAT, CSRRI and Dept. BCPS, Illinois Institute of Technology, Chicago IL

CSRRI
Center for Synchrotron Radiation Research & Instrumentation



What is BioCAT?

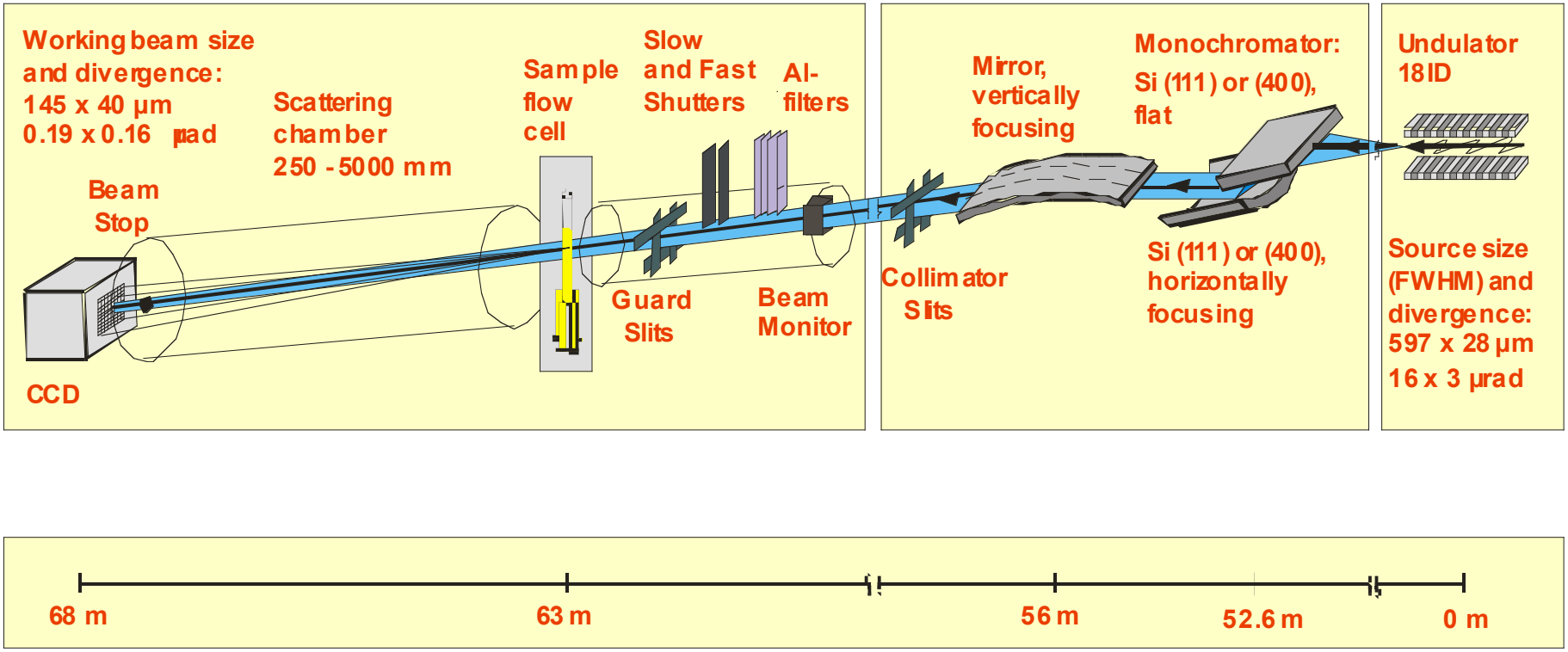
- A NIH-supported research center for the study of partially ordered and disordered biological materials
- Comprises an undulator based beamline, (18-ID) associated laboratory and computational facilities.
- Available to all scientists on basis of peer-reviewed beamtime proposals



Scientific Mission of BioCAT

Modality	**Applications**
Fiber Diffraction	Muscle, Connective Tissue, virus structure, DNA, Amyloids
Solution Scattering	Protein/RNA folding, Protein-ligand interactions
Scanning μ-x-ray florescence microscopy and diffraction imaging	Metal metabolism, neuro-degenerative disease, cancer

SAXS Instrument on the BioCAT 18ID - Undulator Beamline



- **Total X-ray flux 1-2.5 x10^{13} photons/s**
- **Focal spot size ranges from < 50 µm vertical and < 150 µm horizontal to ~3 x 1.5 mm**

Why is Doubly Focused Undulator Radiation Good for SAXS/NCD Studies?

- High flux density for time-resolved applications and obtaining good counting statistics from weakly scattering systems
- Small focal spots along with well-matched high resolution area detectors allow resolving weak diffraction features in the presence of high backgrounds typical of biological samples
- Spatially resolved systems (Micro-diffraction)

Aviex PCCD 16080 Detector

160 x 80 mm

2000 x 4000 39 μm pixels

~5/ADU's/12 keV Photon

~ 1 ADU read noise

~ 1 s readout

Flexible binning modes

Both high sensitivity and high spatial resolution

Pilatus 100K Photon Counting Detector

2D detector, continuous readout till computer disk full

Pixel size	172 x 172 μm^2
Format	487 x 195 = 94 965 pixels
Active area	83.8 x 33.5 mm^2
Counting rate	> 2x10^6 counts/s/pixel
Energy range	3 – 30 keV
Readout time	< 2.7 ms
Framing rate	> 200 Hz

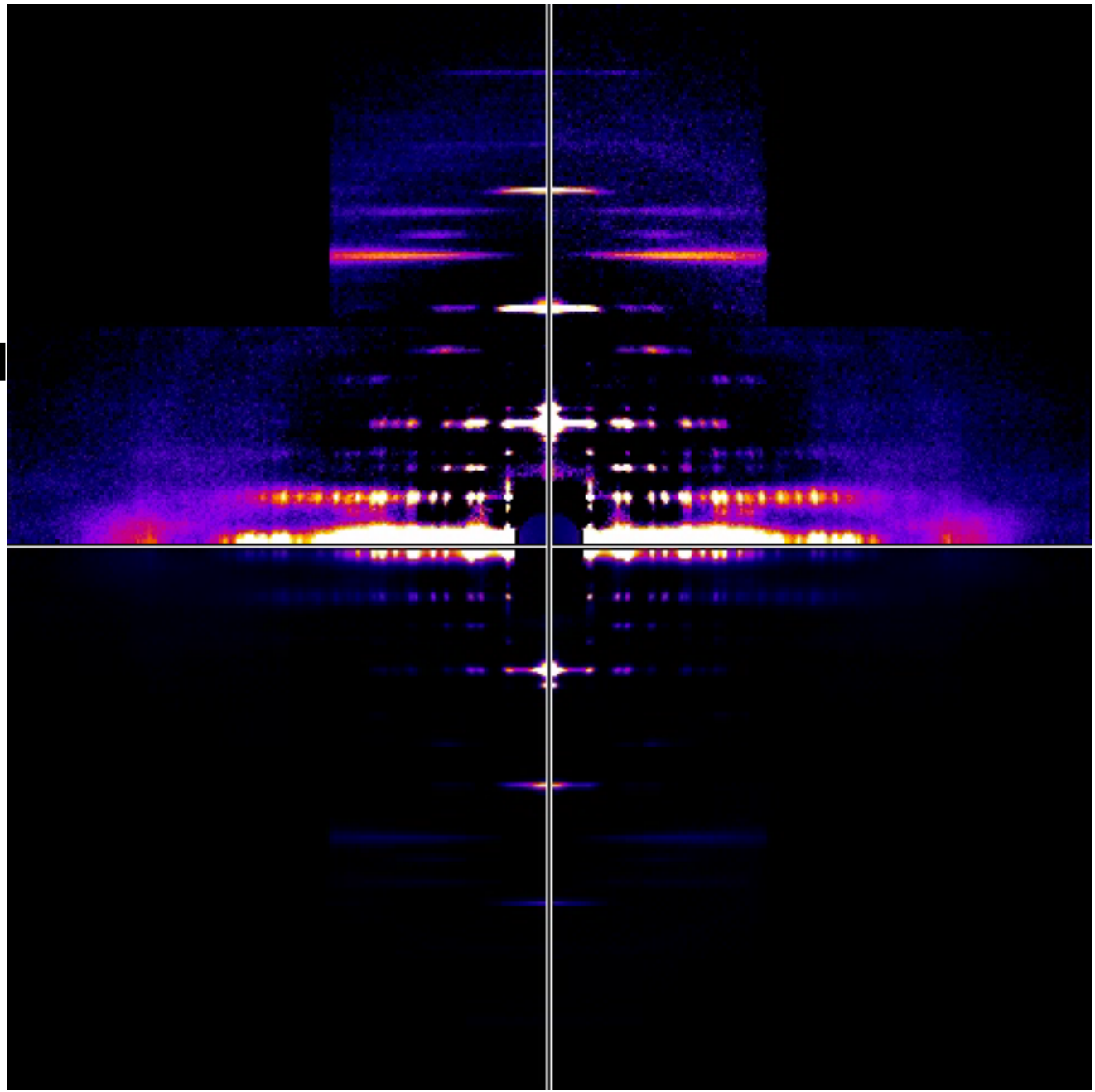
Power consumption 5W, air cooled

Limited Energy resolving ability

200 ns electronic gating possible

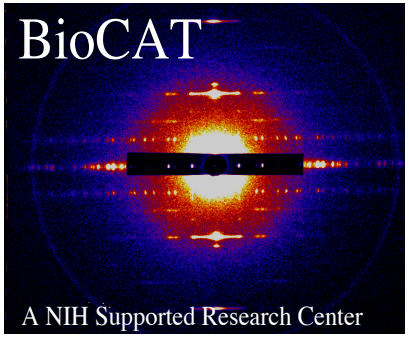
Stretch

Activated



Relax

Low gain



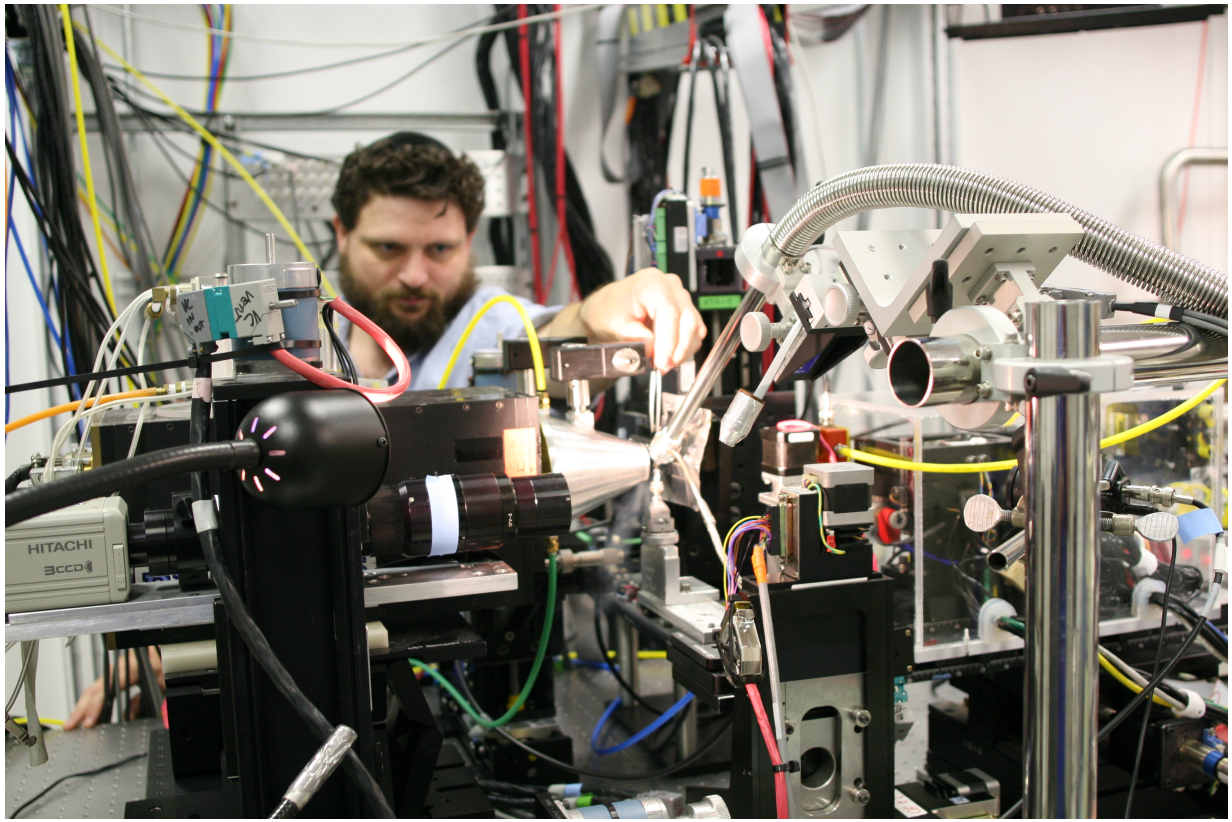
Micro-Diffraction

Minimum focal spot size ~ 5 x 5 μm

Can adjust focal spot position on the fly to optimize flux vs. divergence

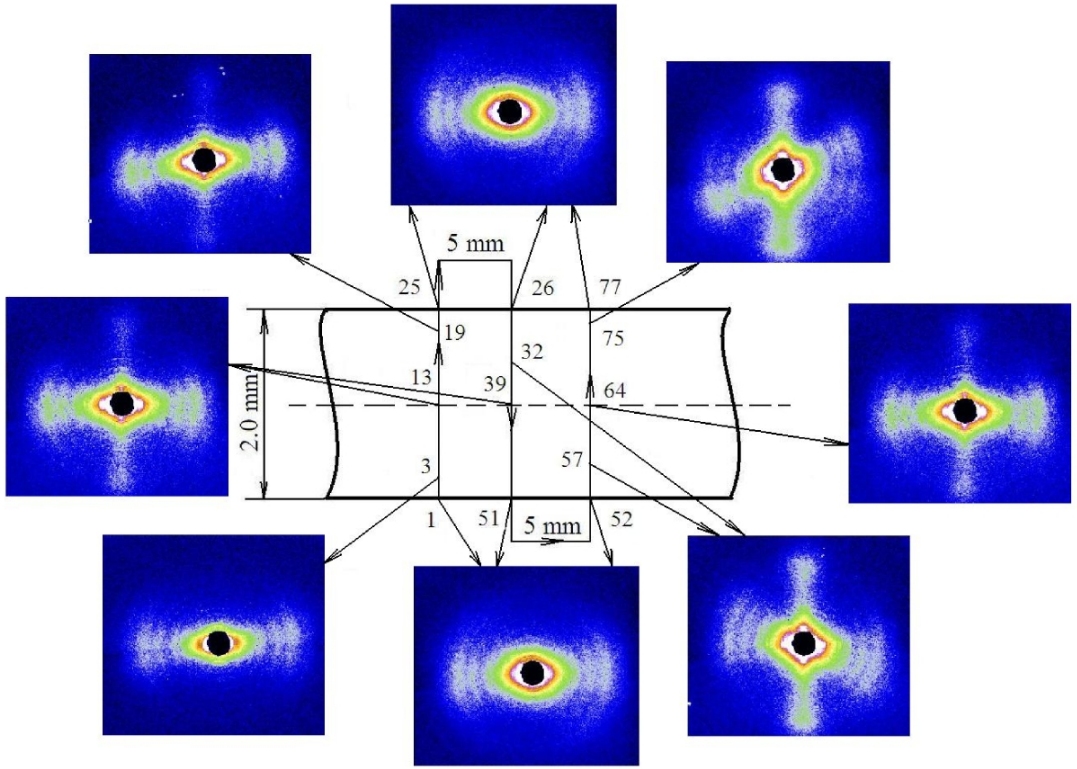
CCD detector with 4000 x 4000 40 μm pixels

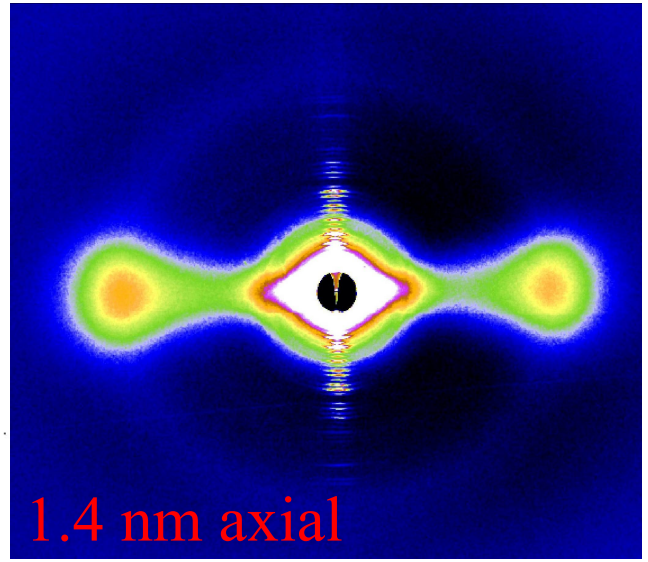
Automated “Diffraction Mapping” possible



Structural Studies of Collagen Type 2 from Lamprey Notochord(Orgel)

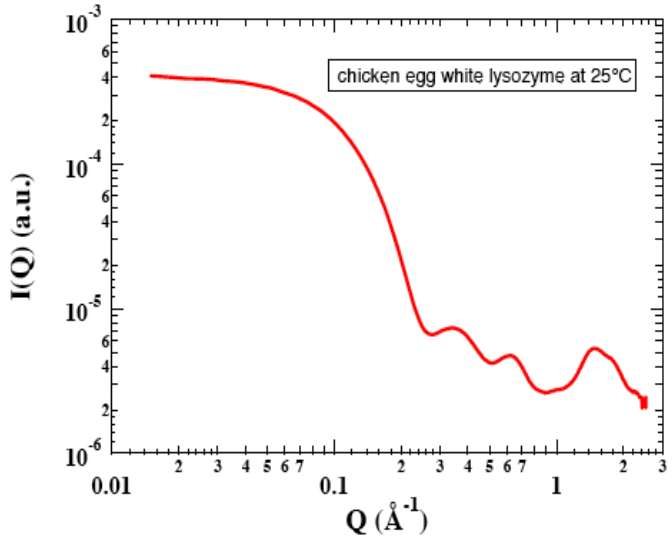
Histology mapping shows where the most crystalline /oriented regions are located

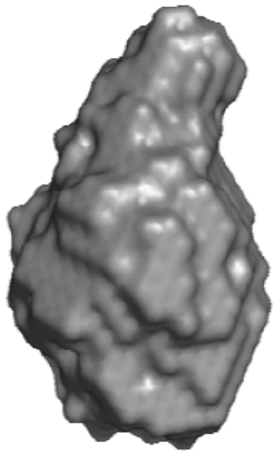




1.1 nm equatorial

Solution Scattering





Combined SAX/WAXS data taken at BioCAT and *ab initio* low resolution structure calculated from the scattering data (~ 8 Å resolution using data up to q=0.8)

Camera lengths from

~ 1 m to 3.5 m

Standard lengths:

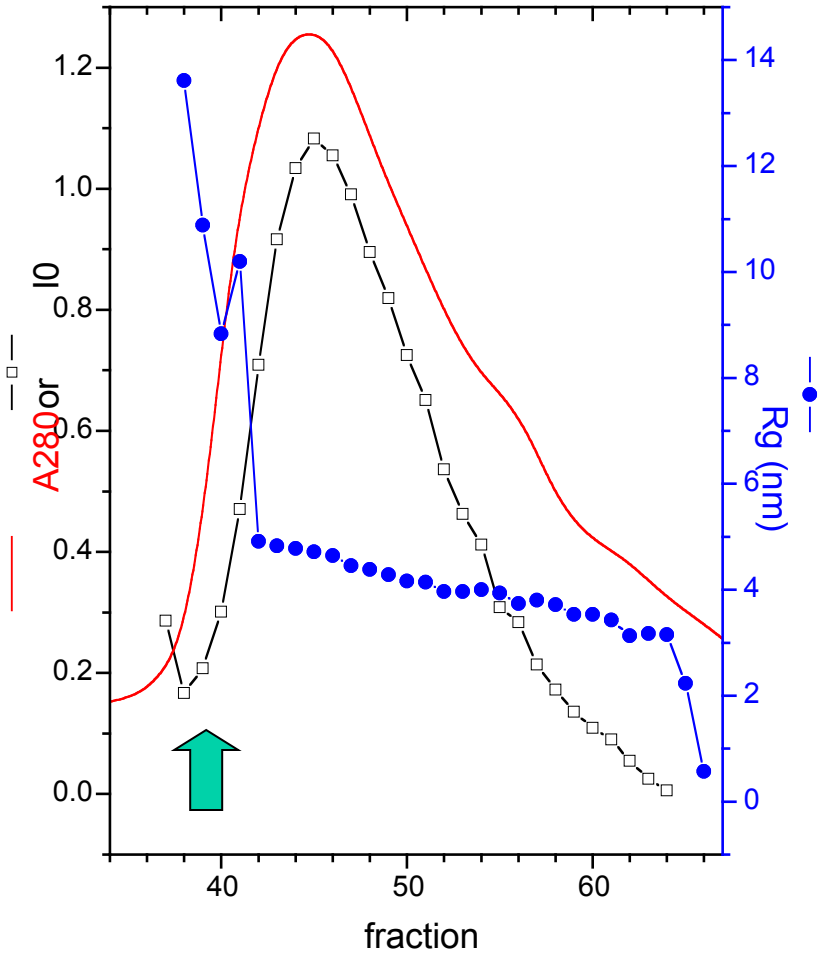
2 m q range:

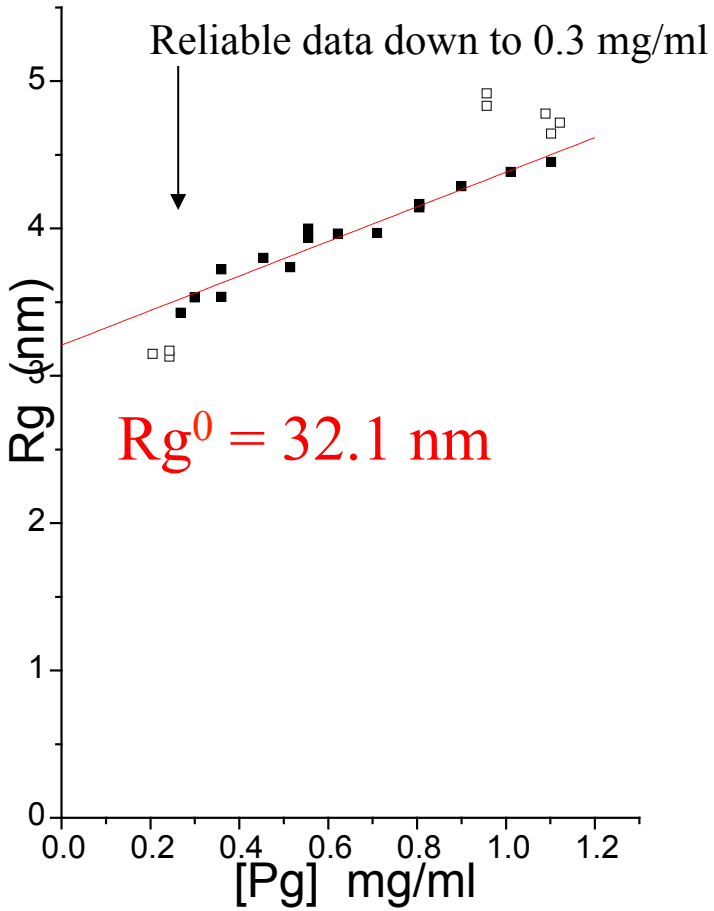
0.007 $Å^{-1}$ to 0.4 $Å^{-1}$

3m q range:

0.005 $Å^{-1}$ to 0.3 $Å^{-1}$

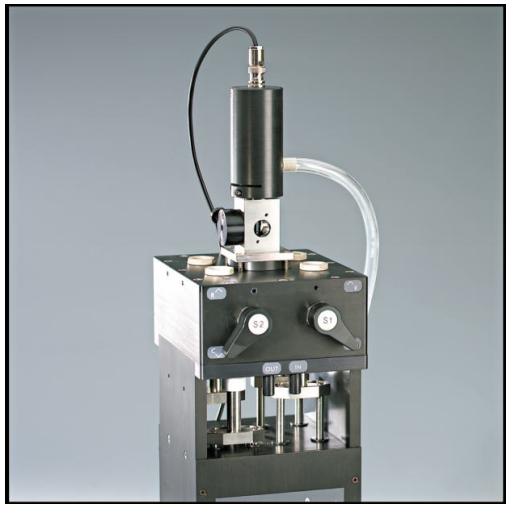
FPLC + SAXS

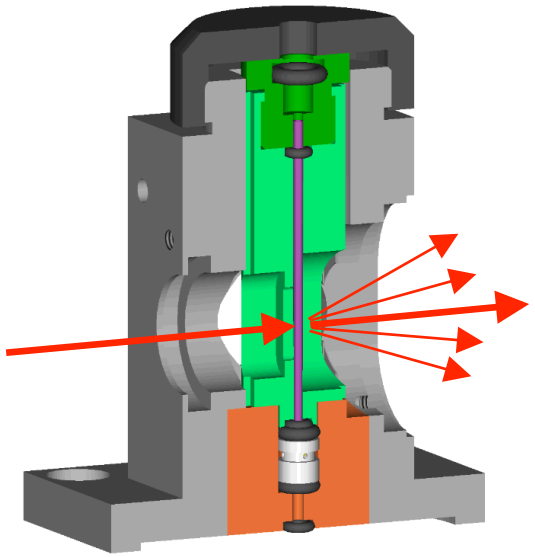




Plasminogen data courtesy N. Menhart, IIT

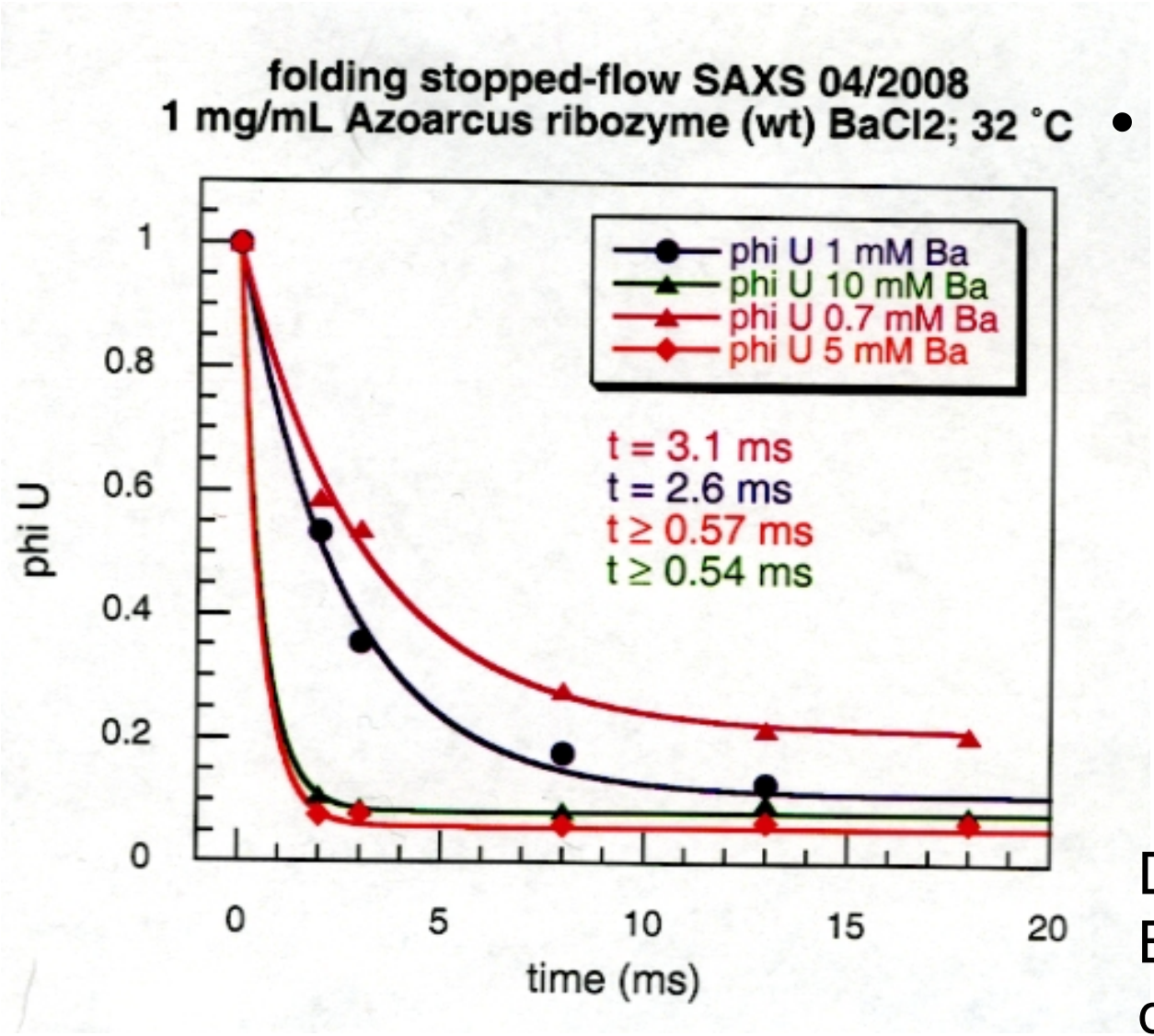
Stopped Flow for Kinetics





- Bio-Logic SFM-400 stopped-flow with new Biologic MEC 22998 microvolume mixer
- ~ 0.5 ms dead time
- Requires flow rate of 8ml/s of sample.
- Typical sample consumption ~ 250 µl (mixed volume) per mixing event

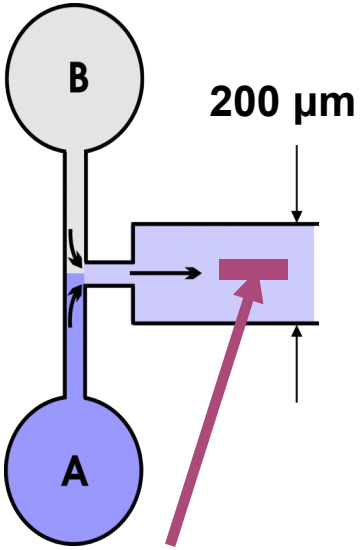
Stopped Flow with Pilatus Detector

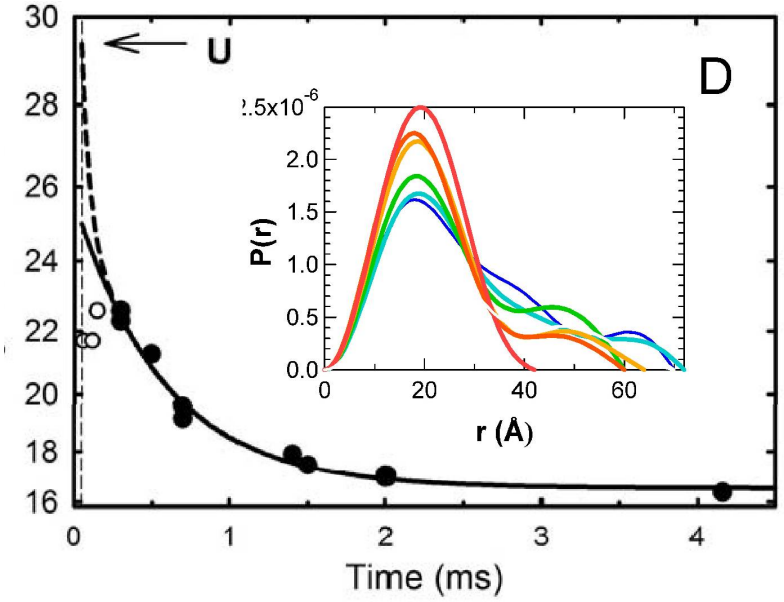


- Continuous SAXS data acquisition with a time resolution of 1 ms with a reaction dead time of 0.5 ms.

Data courtesy of Briber/Woodson group

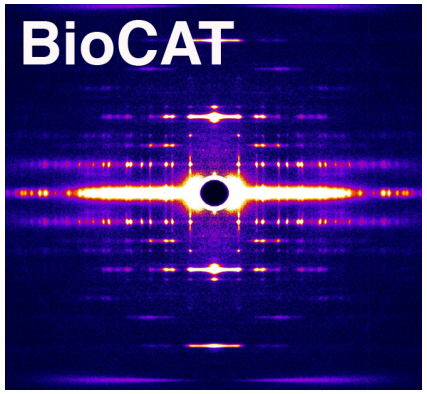
Sub-Millisecond Time Resolved Studies with Turbulent Flow Mixers (Osman Bilsel)





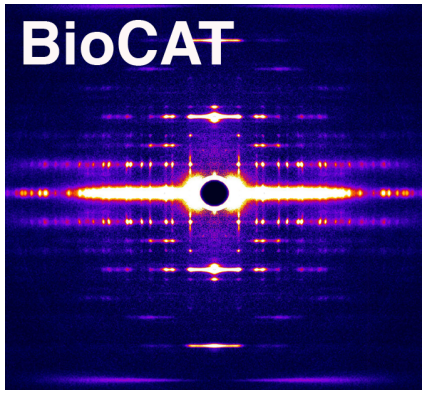
4.5 M→ 0.45 M GdnHCl refolding (with 0.2M imidazole) of cytochrome c

Time resolution : ~80 microseconds



BioCAT Staff

- **Dr. Olga Antipova**
- **Dr. Srinivas Chakravarthy**
- **Mr. Rich Heurich**
- **Prof. Tom Irving**
- **Ms. Clareen Krolik**
- **Prof. Joseph Orgel**
- **Mr. Mark Vukonich**
- **Dr. Weifeng Shang**



BioCAT is supported by the : National Institute of General Medical Sciences 9 P41 GM103622-17

Part of the US National Institutes of Health